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PROTEINASE-INHIBITORY POTENTIAL IN RAT PANCREAS AT THE BURN SHOCK AND TOXEMIA STAGES OF BURN DISEASE

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The increase in general antitryptic activity was by 1.4 times higher for 1 day (at the stage of burn shock), while the total proteolytic activity decreased, compared to the respective parameters of the pancreas in the control group of rats under the conditions of burn disease. Then both indices decrease and remain reduced for 7 days. At the next stage of toxemia (on day 7), with a burn, the total antitryptic activity in the pancreas decreases by 1.6 times compared to the control animals. On the 7-th day, the total antitryptic activity in the pancreas decreases by 2. 2 times, compared to the 1-st day. Changes in proteolytic and antitryptic activity in the rat pancreas cause changes in proteinase-inhibitory potential at the stage of burn shock and the stage of toxemia in experimental burn disease. The proteinase-inhibitory potential of the rat pancreas on the 1-st day after the burn differs from this index value on the 7-th day.

Key words: burn disease, pancreas, antitryptic activity, proteolytic activity.

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ПРОТЕЇНАЗНО-ІНГІБІТОРНИЙ ПОТЕНЦІАЛ У ПІДШЛУНКОВІЙ ЗАЛОЗІ ЩУРІВ НА СТАДІЇ ОПІКОВОГО ШОКУ І СТАДІЇ ТОКСЕМІЇ ПРИ ОПІКОВІЙ ХВОРОБІ

Зростання загальної антитріптичної активності в 1,4 рази відзначено на 1 добу (на стадії опікового шоку), при цьому загальна протеолітична активність падає, в порівнянні з відповідними показниками підшлункової залози контрольної групи щурів в умовах опікової хвороби. Потім обидва показники зменшуються і залишаються зниженими на 7 добу. На наступній стадії токсемії (на 7 добу) при опіку загальна антитріптична активність в підшлунковій залозі знижується в 1,6 рази, в порівнянні з контрольними тваринами. На 7 добу загальна антитріптична активність в підшлунковій залозі знижується в 2, 2 рази, в порівнянні з 1-ю добою. Зміни протеолітичної і антитріптичної активності в підшлунковій залозі щурів викликають зміни протеїназно-інгібіторного потенціалу на стадії опікового шоку і стадії токсемії за експериментальної опікової хвороби. Протеїназно-інгібіторний потенціал в підшлунковій залозі щурів в 1-у добу після опіку відрізняється від даного показника на сьомий день.

Ключові слова: опікова хвороба, підшлункова залоза, антитріптична активність, протеолітична активність.

The work is a fragment of the research project "General patterns of pathological changes in experimental burn disease and development of methods for its correction", state registration No. 0119U102850.

From our previous studies in rats, changes in the inhibitory potential of the blood serum as well as in the pancreas in acute stress are known. The total proteolytic activity both in the blood serum and in parallel in the pancreas, increases against the background of total antitryptic activity declining [1].

The literature highlights the results of proteinase-inhibitory potential studies in stress under the burn disease conditions [2].

Proteolytic activity is known to be different in the blood at the initial and final stages of burn disease. The maximum peak in rats occurs after 12 hours. Also increase is observed on days 1 and 7. Decrease in the proteolytic activity is registered from the 7th to the 28th day (28 days). [1].

Literature data are known on the decline of antiproteolytic activity.

With a burn, the maximum decrease in the blood is registered in rats on the 1st day almost by 4 times [1].

Some authors have described studies of changes in proteinase-inhibitory potential in other organs of rats. In the lungs there was an increase of total proteolytic activity after burns (days 1, 7) [2]. But the antitryptic activity first increases (day 1), then decreases (days 7 – 28) [2].

In the kidneys of rats, under the conditions of burn disease, studies indicate the changes that cause an imbalance in the proteolysis – proteolysis inhibitors system [3].

Our previous experiments on the pancreas show differences in the proteolytic activity in burns. At the first stages (days 1, 7) a decrease occur, in the following stages (21 days (21 days), 28 days) – an increase in the index takes place [1].

There are no data in the literature on the study of total antitryptic activity in the pancreas of rats with burn disease. Features of proteinase-inhibitory potential in the animals' body at the stages of burn shock and toxemia are not described. Therefore, it is advisable and relevant to determine the antiproteolytic activity in the pancreas of rats with burns, because it will permit to analyze the features of proteinase-inhibitory potential at the initial stages of burn disease.

The study of this issue is important because activation of proteolytic enzymes and a parallel decrease in the activity of these enzymes' inhibitors can lead to toxic effects. Proteolytic enzymes, acting on tissues, cause increased protein breakdown, which leads to the increased urea and creatinine contents in the blood. Toxic products contribute to the development of intoxication syndrome in the body [4].

The purpose of the work was to study the total antitryptic activity in experiments on the pancreas of rats after burns (days 1, 7) and to analyze the proteinase-inhibitory potential.

Materials and methods. Experiments were carried out with 15 male rats (180 - 250 g). Recommendations set out in the European Convention were observed. Rats were selected from those kept under standard vivarium conditions on the general diet. Development of burn disease was induced in compliance with the known method. The essence of the method by A.P. Dovgansky is that the epilated skin surface of the hind limb in the experimental group animals is immersed into water with the temperature of +70 - + 75^{o} C, under light ether anesthesia, the immersion lasting 7 seconds. Under these conditions, a burn of III A - B degree appears as a standard model of the burn disease development in the experiment, according to modern ideas.

Animals were killed after burns (on the 1st and 7th days) under ether anesthesia.

Total antitryptic activity was determined by the weight of inactivated trypsin.

The method described by Veremeenko K.N., Goloborodko O.P. Kizim A, in the manual "Proteolysis in normal and in pathology" is based on determining the difference between the activity of a sample containing a certain amount of trypsin, and the activity of the sample, in which part of the enzyme binds to pancreatic tissue inhibitors.

Homogenate was prepared of the rat pancreas. A working solution containing the enzyme was added to the organ tissue homogenate. An enzyme-inhibitor complex was formed in the mixture. Casein was added to the mixture and then incubated. The reaction was stopped by adding a solution of trichloroacetic acid at the appropriate stages for control and experimental samples. Centrifugation was performed. The optical density of the obtained clear solution was determined. A calibration graph for trypsin was prepared. The enzyme activity was determined according to the calibration schedule.

To study the proteinase-inhibitory potential in the pancreas, proteolytic activity was determined in the respective tissue homogenates of rat organs in burn disease. The total proteolytic activity in the body was determined by the increase in free amino nitrogen content. The source of its formation were proteins that were to be reduced by the enzyme. The principle of the method is based on the interaction of amino acids with ninhydrin solution and formation of the blue colored reaction product. The color intensity of the solution is directly proportional to the amino acid content.

Mathematical and statistical analysis of the obtained results was performed, determining the arithmetic mean, standard deviation, mean error of the mean. The Student's criterion (t) was determined to assess the reliability of the identified differences [5]. The condition for the application of Student's criterion to compare the mean values was the normality of the studied values distribution in each of the compared groups.

Results of the study and their discussion. The activity of enzymes, which were determined in the pancreas of intact rats was considered as 100%.

Fig. 1, 3 illustrate the results of studies in experimental burn disease of rats, which show that the total antitryptic activity rises (on the 1st day – by 1.4 times) compared to the index of the pancreas in the intact group of animals.

Fig. 2 and 3 show the results obtained in the study of the burn disease impact on rats, which show the following: the total antitryptic activity falls (on the 7th day) – by 1.6 times lower than the pancreas control, and if compared to the 1st day – by 2.2 times lower on the 7th day than on the first day.

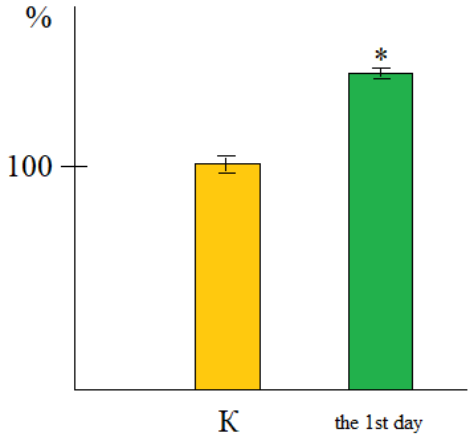

Fig. 1. The total antitryptic activity of the pancreas at the stage of burn shock in rats, M ± m: K - intact group; the 1st day – a group of animals that were subjected to burn and examined after 1 day; * p <0.001.

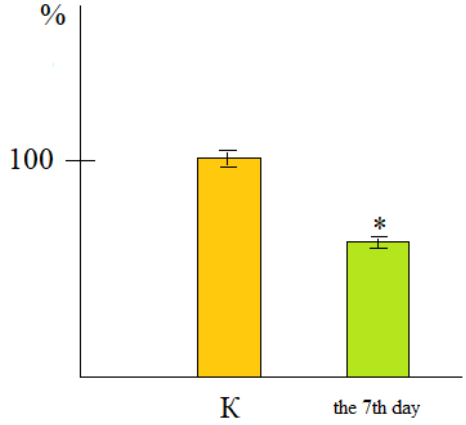

Fig. 2. The total antitryptic activity of the pancreas at the stage of toxemia of rats after burn, M ± m: K - intact group; the 7th day – a group of animals that were subjected to burn and examined after 7 days; * p <0.001.

Fig. 3 summarizes the results of experiments on rats with burn disease, which show that on the 1st day in the pancreas, against the background of increased total antitryptic activity, a decrease in proteolytic activity is observed.

On the 7th day with a decrease of total antitryptic activity in the pancreas (fig. 3) proteolytic activity remains reduced as well.

Thus, as it can be seen in fig. 3, in the conditions of burn disease, proteinase-inhibitory potential in the rat pancreas differs on the 1st day (at the stage of burn shock) from this index on the 7th day (at the stage of toxemia).

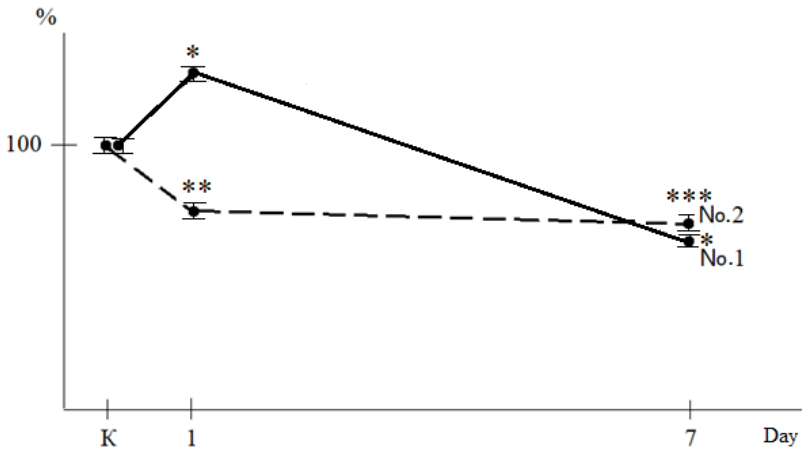

Fig. 3. Total antitryptic activity (No. 1) and total proteolytic activity (No. 2) of the pancreas in burn disease, M ± m: K – intact group; the 1st day – a group of animals examined 1 day after the burn; the 7th day – a group of animals examined 7 days after the burn; * p <0.001; ** - p <0.05; *** - p <0.1.

It is possible that the expediency of increasing proteinase inhibitors in pancreatic tissues on the 1st day after the burn is that they can inhibit the activity of proteolytic enzymes in the pancreas and reduce the proteolytic activity in the pancreas on the 1st day after the burn by 1.4 times, which we reported in our work (fig. 3).

It is possible that proteolytic enzymes flow from the pancreas into the blood, where on the 1st day there is an increase in total proteolytic activity [1]. Therefore, it is especially important to increase the total antitryptic activity in the pancreas on the 1st day against the background of reducing the total antitryptic activity of the blood by 3.75 times [1].

There is evidence in the literature that stress increases the activity of proteolytic enzyme inhibitors, possibly due to their synthesis. [6].

Peak increase parallelism of the total antitryptic activity on the 1st day is observed in the lungs [2].

In the pancreas, various enzymes are formed in the inactive form. [7]. Synthesis of the inactive form of proteinases is of particular biological importance, as it prevents damage to the secretory cells of the tissues

in which they are synthesized, i. e. prevents self-digestion. Proteolytic enzymes synthesized in the pancreas are secreted to the duodenum as inactive protease precursors.

Proteolytic enzymes (proteases) are produced by cells of the mucous membrane in the digestive tract and exocrine part of the pancreas and are involved in the digestion of proteins.

The proenzyme trypsinogen is formed in the pancreas, enters the intestine, is activated by enterokinase, and the active enzyme trypsin is formed at pH 7.5 - 8.0.

Enterokinase

Trypsinogen – trypsin + hexapeptide.

Trypsin is an endopeptidase that most actively cleaves peptide bonds formed by the basic amino acids – arginine and lysine [7].

Proenzyme chymotrypsinogen is formed in the pancreas, enters the intestine, is activated by trypsin, and the active enzyme chymotrypsin is formed.

Trypsin

Chymotrypsinogen – chymotrypsin + inhibitory peptides.

Chymotrypsin is an endopeptidase that cleaves 50% of peptide bonds in protein and food peptide molecules, including pepsin- and trypsin-insensitive bonds. Chymotrypsin has a broader substrate specificity than trypsin. It is manifested in the fact that chymotrypsin catalyzes not only peptides, but also esters, amides and other acyl derivatives.

Short peptides formed under the action of endopeptidases are subject to the action of exopeptidases [7].

Proenzymes of procarboxypeptidase A and B are formed in the pancreas, enter the intestine, are activated by trypsin, and active enzymes of carboxypeptidase A and B are formed.

Carboxypeptidases are peptidases that hydrolyze peptide bonds formed by C-terminal amino acids in peptides (with a free carboxyl group).

Carboxypeptidase A cleaves amino acid with a hydrophobic radical (alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan) from the C-terminus of the peptide.

Carboxypeptidase cleaves the amino acid residues of lysine and arginine from the C-terminus of the peptide.

The proenzyme proelastase is formed in the pancreas, enters the intestine, is activated by trypsin, and the active elastase enzyme is formed.

Elastase is an endopeptidase that cleaves the connective tissue protein elastin, namely peptide bonds formed by residues of small amino acids – glycine, alanine, serine.

The proenzyme procollagenase is formed in the pancreas, enters the intestine, is activated by trypsin, and the active enzyme collagenase is formed.

Collagenase cleaves the connective tissue protein collagen.

Aminopeptidase intestinal enzymes complete the cleavage of peptides to dipeptides. In their turn, dipeptides are cleaved by intestinal enzymes dipeptidases to free amino acids.

Trypsin is formed in the pancreas as well as other proteinases in the inactive form. The physiological essence of this phenomenon is important, because the active form of trypsin would perform a proteolytic action aimed at destroying organs, cells, enzymes.

There exists one more defense mechanism for the pancreas. It is that the body provides itself with the synthesis of pancreatic trypsin inhibitor. It is a low-molecular peptide. Its mechanism of action is that the inhibitor is able to strongly bind to trypsin and chymotrypsin, due to their active centers. Thus, it causes their inhibition.

Various factors affect changes in the activity of both proteolytic enzymes and their inhibitors, and hence, changes in proteinase-inhibitory potential.

Under the influence of various factors, including stressors, such as burns, their activity in the organ's tissues can change. Changes in proteinase-inhibitory potential in the pancreas and activation of proteinases that cleave various proteins are dangerous, because they can disrupt the processes of regulation and metabolism in the body, blood, etc.

Uncontrolled activation of trypsin causes damage to the tissues of the pancreas, which leads to self-digestion of the organ's tissues. Trypsin activates other pancreatic zymogens, such as proelastase, procarboxypeptidase or profospholipase A_2, which damage acinar cells. This may be due to the exhaustion of the inhibition mechanisms (inhibitors and others) as a cause of damage to the pancreas [8].

Therefore, it is advisable to detect an increase in the activity of proteinase inhibitors and a decrease in the total proteolytic activity in the pancreas at the stage of burn shock (the 1st day).

It is possible that on day 7, the total antitryptic activity is sufficient to inhibit proteolytic enzymes in the pancreas. Perhaps there is a depletion of the organ and the protective system of antiproteolysis on the 7th day.

Therefore, already on the 21st and the 28th day the total proteolytic activity in the pancreas increases [1].

The peak parallelism of decrease in the total antitryptic activity on the 7th day is observed in the lungs [2].

Disorders of proteinase-inhibitory potential in the pancreas can cause disorders and imbalance of protein metabolism in the organ, blood and the body as a whole and play a role in the burn disease pathogenesis.

Increased activity of proteolytic enzymes in the blood already at the burn shock stage causes the protein breakdown at this stage of burn disease, increases at the next stage, which is one of the causes for the burn exhaustion development.

Changes in protein metabolism after burns towards the catabolic processes lead to development of a negative nitrogen balance as a consequence of protein metabolism disorder. As a result, residual nitrogen accumulates in the blood [4].

In burn disease, increased protein cleavage leads to the accumulation of toxic substances.

Inhibitors of proteolytic enzymes normalize metabolism and reduce proteolysis in the pancreas at the early stages of burn disease. Therefore, it is advisable to use and introduce protease inhibitors to reduce the effects of proteolysis and for treatment of burns.

Thus, the study of proteinase-inhibitory potential in the pancreas, blood, and other organs permits to study the mechanisms of burn disease, the causes of protein and other substances metabolism disorders, burn exhaustion.

Conclusion

The proteinase-inhibitory potential of the pancreas differs from this index in the lungs and blood.

Proteinase-inhibitory potential in the pancreas varies depending on the stage of the burn disease. At the stage of burn shock (the 1st day) the total antitryptic activity increases, and proteolytic activity is reduced. And at the stage of toxemia (the 7th day) the antitryptic activity decreases in the pancreas, and proteolytic activity remains reduced. This response of the pancreas can reduce proteolysis at the stage of burn shock and the stage of toxemia.

Prospects for further development in thisfield lie in the fact that it is planned to study the general antitryptic activity in the pancreas at other stages of burn disease.

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